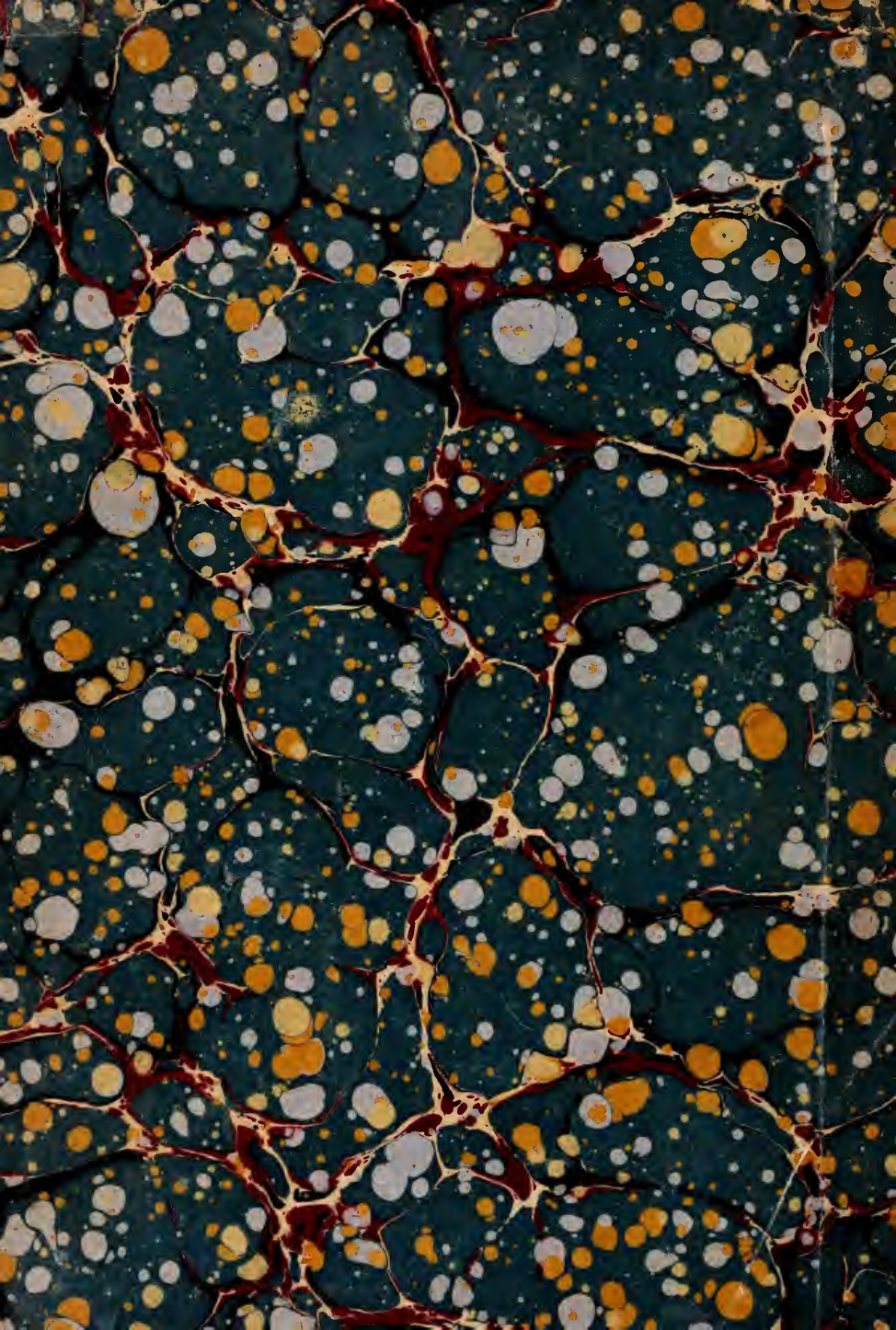
MODERN THEORIES OF BACTERIAL IMMUNITY

HAROLD C. ERNST, M.D.



E. G. Gardner

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INTRODUCTION.

The following pages are an abstract of a short series of lectures delivered by request to the class of 1905 in the Harvard Medical School, in January, 1903. The method of illustrating Ehrlich's theories is given in an attempt to make this obscure subject somewhat more clear, and the glossary of terms may prove useful to beginners.

Modern Theories of Bacterial Immunity.

I.

Immunity in the bacterial infectious processes is divided into:

Natural Immunity. — By this term is meant the ability to resist the invasion of infectious agencies, which is often found under natural conditions, *e.g.*, the resistance of many races of animals to infection with typhoid fever, or the resistance of the human race to hog cholera; and

Acquired Immunity. — This form of immunity is said to occur when the condition of resistance appears, following an attack of and recovery from an infectious agent. It is manifest in persons or animals that have recovered from many forms of such invasion, or it may appear as the result of measures purposely taken in order to favor its development. In

this case it may be spoken of as an artificial immunity, and such artificial immunity has been obtained in a number of different ways.

Acquired immunity, as seen following the action of bacterial infectious agents, is again divided into the forms of Active and Passive Immunity, and the distinction between these two is of great importance in understanding many of the problems arising in connection with the study of immunity in general.

Active acquired immunity occurs in the course of recovery from an infection, or may be produced artificially. In the latter case it is the result of the injection of gradually increasing doses (amounts) of the toxic products of micro-organisms, beginning with quantities much less than the minimal lethal dose, and increasing until there is reached a condition of resistance without reaction to many times such minimal lethal dose. Such a condition may be obtained by the injection of the bodies of bacteria killed by the application of heat; of the bodies of bacteria in the living condition, but whose virulence has been diminished in various ways; or by the use of

filtered culture fluids in which bacteria have grown and produced soluble poisonous products.

Examples of the first method are the procedures in securing immunity against typhoid fever, cholera, and plague; of the second, the first and second "vaccines" of anthrax; of the third, the use of the soluble toxines of tetanus and diphtheria.

Passive acquired immunity. — It has been found that in certain cases of active immunity produced as above, there appears coincidently with the complete resistance to the injection of the toxic material a substance, existing in the blood-serum of the animals so treated, that has an antagonistic action to the toxic material, both in the test-tube and in the living animal tissues. This substance is spoken of as "antitoxine," and with the serum containing it is used for the protection of tissues invaded by the toxine-producing bacteria; as in the therapeutic use of diphtheria antitoxine.

Such antitoxines occur in the blood-serum of animals attacked by, or immunized with the toxic products of tetanus and diphtheria — the cause

of both of these diseases being bacteria that produce extra-cellular or soluble toxines.

They do not occur, or only to a very limited extent, in animals attacked by or immunized with the toxic products (bacteria) of typhoid fever, cholera, or plague; the cause of these diseases being bacteria that produce intracellular or non-soluble toxines.

When they do occur and are used for protective purposes, the condition following their use is spoken of as Passive Immunity.

The differences between these two forms of immunity are very marked.

Active immunity is slow in appearing, is more or less dangerous to secure, is always attended with at least some discomfort, but is very lasting when once attained. A horse brought to a high degree of immunity to diphtheria toxine will show a marked degree of such immunity for a very long time — extending over months and even years.

Passive immunity, on the other hand, is very rapid in its appearance, is attended with no

danger and practically no discomfort, but is limited in duration and disappears very rapidly. An animal protected against a dose of diphtheria toxine by the injection of antitoxine (of diphtheria) is protected only against that dose of toxine, and is susceptible to a second dose in a very short time.

Attempts to produce a condition of artificial immunity to infections of various kinds have been made for a very long time, and there exist accounts of such attempts among savage tribes — the beginning appears to have been in the direction of protection against the bites of venomous serpents.

The most well-known efforts to secure artificial immunity, and before the present knowledge of the bacteria was developed, were the inoculations against small-pox — a knowledge of the beneficial effects of which existed in Asia certainly for generations before its introduction into England by Lady Montague. These inoculations consisted in the use of contents of the pustules in mild cases of small-pox, which were introduced through scarifications of the skin or

mucous membranes of well persons, in the hope of producing a mild case, and thus protecting the individual from an attack of a more malignant form of the disease.

Jenner's application of his observation in Gloucestershire, where he was born, that dairy-maids handling cows sick with cow-pox appeared to be protected against small-pox was the beginning of the stamping out of this great plague in all parts of the civilized world.

The discovery of the possibility of the attenuation of the virulence of a culture on the one hand, and of the possibility of securing an artificial immunity with such attenuated cultures on the other, were more or less the result of chance. Pasteur, with Chamberland and Roux, had been studying the bacillus of chicken-cholera, and interrupted their work during the vacation season of 1879. Upon attempting to resume it they found that their cultures, kept over during the summer, still retained their vitality, but had lost their virulence, either wholly or in great part. (Valery-Radot. La vie de Pasteur, p. 427.) Testing the effects of such cultures led

Pasteur to the steps resulting in the practical application of the laboratory results in such procedures as the protective inoculations against anthrax and rabies. (See C. Rendus de l'Academie des Sciences, 1880, T. XC., pp. 936, 952, 1030; T. XCI., pp. 571, 673.)

The statement of the general principles was at first met with much opposition and incredulity, but was so overwhelmingly confirmed by other observers that such incredulity soon disappeared.

The first theory that was advanced to explain the phenomena seen was that of Pasteur himself, and was known as The Exhaustion Theory. In accordance with this, the condition of immunity following the injection of attenuated cultures of a bacterium may be supposed to be due to the exhaustion of certain elements in the tissues used by the bacteria during their growth. These elements are not resupplied for some time by the tissues, and during that time fresh bacteria entering the tissues, finding the absence of something necessary for their growth, cannot grow, and therefore no infection can take place. Such a condition of things apparently exists in the test

tube; for if a fluid in which a micro-organism has grown be filtered and planted with a fresh culture, no growth will occur. This certainly might be due to the exhaustion of the nutrient medium, and that it is so seems to be shown by the fact that an addition of a very small amount of fresh medium is followed by a profuse growth of the bacterium in question. The same thing was supposed to occur in the living tissues.

Such a theory, however, would not account for the facts seen in Natural Immunity, in which no previous growth of the bacteria could be supposed to occur. Nor would it explain the results seen in Algerian sheep. These animals are not susceptible to inoculation with anthrax bacilli sufficient to kill ordinary French sheep, but will succumb to very large doses of such bacilli. If, then, the first resistance was due to the absence of nutrition for a comparatively small number of bacilli, how could a much larger number find enough to live upon?

In view of such objections as these, Chauveau brought forward his Retention Theory to explain the phenomena seen. In accordance with this,

the bacteria may be supposed to elaborate a material that is hurtful to themselves, and this substance is retained in the tissue cells or fluids for some varying length of time, during which time a fresh invasion of bacteria would be followed by no results. To explain such cases as those of the Algerian sheep by this theory — in which the sheep are not susceptible to attack by small doses of anthrax bacilli, but are susceptible to overwhelming doses — it would be necessary to suppose the existence of a fixed amount of the opposing substance which would be more than neutralized by the excessive doses of the anthrax bacilli, after which infection occurred. Although Chauveau objected to the exhaustion theory, that it did not explain natural immunity, his own supposition did not do so any better. Nor did it serve to explain more than a very small part of the experimental facts observed. Pasteur himself furnished one instance — that of chickens that are not susceptible to anthrax at their normal temperature, but become so when this temperature is lowered. His comment upon this fact and its bearing upon Chauveau's theory was that it could not be supposed that a

preventive substance existed that would disappear under the influence of cold.

Buchner brought forward a theory to explain the fact of obtaining immunity in the infectious processes. It was based upon the then accepted belief (1877–1883) that a bacterium produced its effects in a localized fashion (that the pneumococcus, for example, could produce effects only in the lung), and also that the inflammatory reaction occurring at the site of growth of the bacteria represented a reënforcement of the local tissues, by the general system. So soon as evidence accumulated that the action of very few bacteria is limited in the way suggested, it became clear that this theory was not sufficient to explain even a small part of the facts observed.

Various other partial explanations were offered, each lasting only so long as the rapidly growing results of experimentation needed to show their limitations.

The only ones that have withstood the test of time are those of Metchnikoff, representing the *cellular* theory, and of Ehrlich, representing the *humoral* theory.

II.

Metchnikoff's work is of the most remarkable kind; painstaking and laborious to an extreme degree, he has for more than twenty years put forth a series of papers that are unsurpassed for the closeness of reasoning and fertility of device used to demonstrate his points. No account can be so satisfactory as his own, and the present summary of his theory of the phagocytic action of the tissue cells in securing immunity is largely paraphrased from his own words. (Metchnikoff. L'Immunité. Paris, 1901, pp. 541 *et seq.*)

He began his studies on the germinative layers, and was able to explain satisfactorily to himself the part played by the ectoderm and the entoderm. While working in this direction his attention was attracted to the intracellular digestion occurring in many of the lower animals, and he was led to consider this property as one common to the stock from which are derived all present known types of the animal

kingdom (except protozoa). Considering the ectoderm as the source of the covering of all primitive polycellular animals, and the entoderm as the source of the organs of digestion, the part of the mesoderm remained a mystery until certain of his studies on sponges led him to think that perhaps this layer might have acted in the hypothetical primitive animals as a mass of digestive cells, in every respect like those of the entoderm. Such a theory necessarily attracted his attention to the property that these mesodermic cells have of seizing upon foreign bodies.

The fact had been known for a long time, both in respect to the power of the white corpuscles of the vertebrates to seize upon foreign bodies and of the ameboid cells to seize upon particles of coloring matter. But no one had looked upon this property as one of digestion, and it had even been considered as simply a passive action. Metchnikoff's studies upon sponges and some of the simpler forms of sea animalculæ convinced him that the presence of foreign bodies in the ameboid cells of the mesoderm should be interpreted as an *active* englobement,

and that in all its aspects it resembled very closely the phenomena of intracellular digestion going on in the epithelial cells lining the digestive tract of very many of the inferior animals. At Messina, in 1882–1883, he set himself to secure definite evidence of this fact, and did so by proving that these cells (of the mesoderm) seize upon foreign bodies of very varying nature by their living prolongations, and that some of these foreign substances undergo a true digestion in the interior of these ameboid cells.

Then the idea suggested itself to him that this digestive function, evidently fixed in the mesodermic cells, might also play a part in many of the vital processes of the animal. Working from this point of view, he was able to demonstrate that during the complicated metamorphosis of echinoderms, such as the synaptera, the mesodermic ameboid cells played a part in the atrophy of many embryonic organs.

He had never studied medicine, but he was struck at one time by an exposition by Cohnheim of the facts of the latter's theory of inflammation. The facts as presented impressed him strongly,

especially that of the emigration of the leucocytes, but the theory made to fit the facts did not appeal to him, and he saw at once that study of inflammation among lower animals of the simplest type and organization would certainly throw light on this process in the vertebrates, as well as in the frog — the animal upon which Cohnheim's experiments had been made.

Since, in the atrophy of the larval organs of the synaptera, the essential part is played by the ameboid cells of the mesoderm which collects in masses there, possibly the inflammatory exudates manifest some especially important function by their richness in white corpuscles. So that it suggested itself to him to introduce splinters through wounds under the skin of transparent marine animals; if the supposition was correct there should appear a collection of ameboid cells at the point of irritation. Choosing "bipinnaires," — the large embryonic forms of star-fish, — he stuck rose thorns in them, and these were seen to be at once surrounded by masses of ameboid cells, exactly as in man after the introduction of a splinter or other irritant substance. In other words, the first point was

determined, that an inflammatory exudate must be considered as a reaction against all sorts of lesions, and that exudation is a primitive phenomenon, older than the nervous system or the blood-vessels.

At the time these researches were going on (1882), the general theory in regard to inflammation was that it was, at least in most part if not wholly, the result of bacterial action. As a result of this belief, the conclusion was reached that diapedesis and the gathering of the white corpuscles in inflammatory disease was to be looked upon as a means of defence of the tissues against the bacteria, and that the leucocytes served to englobe and destroy them. Upon such a hypothesis, the explanation of inflammation becomes at once most clear and simple, and it was such an explanation that Metchnikoff set himself to prove, if possible.

The general belief of the pathologists at that time was that the bacteria found a favorable location in the leucocytes and were carried about by them instead of being destroyed. So that to secure recognition for his theories he was obliged to overcome the then

belief of all but a few of the specialists in that branch of medicine.

His effort was to develop the idea that cellular digestion in unicellular organisms had been transmitted by heredity to the superior animals, and is preserved in the mesodermic ameboid cells. These cells being able to englobe and digest all sorts of histological elements may well be able to apply the same power to the digestion of the bacteria — and that they are able to do this he demonstrated in many cases by introducing bacteria into lower animals and watching their englobing and destruction by the ameboid elements. It was evident that the simple demonstration of this occurrence was not sufficient for the development of the general theory, and he went on to find diseases in the lower animals that were illustrative of the same thing. This he succeeded in doing in the case of the daphnia, — small crustacea common in fresh water, — and in them followed out and watched an actual struggle between their leucocytes and the spores of a blastomycete; on the one hand it often happened that the cells were successful in

protecting the body against the attacks of the spores, and on the other the spores overcame the action of the leucocytes and overwhelmed the tissues. (Virch. Arch., 1884, T. XCVI., p. 177.) Some time after this he published his work on anthrax (Ibid., 1884, T. XCVI., p. 502), in which he demonstrated in the case of the vertebrates, also, that the bacteria penetrated the cells and set up a definite reaction between themselves and these cells.

As a result of these investigations, Metchnikoff elaborated his theory of phagocytosis against bacterial invasion — based upon the power of the ameboid cells to seize upon and destroy bacterial cells, thus preventing them from developing and injuring the tissues.

He goes on to say (*l. c.*) that he had supposed that the facts of absorption and in regard to the leucocytes already accumulated would lead the pathologists to look favorably upon the theory that these leucocytes were active defenders of the system against the attacks of the bacteria. This, however, was far from being the

case, and the most active opponents of the theory were those he had supposed would find reason to support it.

Baumgarten was the first of these and perhaps the most important, his most forcible theoretical objection being that just when the danger is the greatest the leucocytes are conspicuous by their absence, and that this is a fatal objection to any assumption that they can play an active opposing part to the growth of bacteria in the tissues. Metchnikoff devoted several years (Virch. Arch., 1888, T. CXIV., p. 465; Ann. de l'Institut Pasteur, 1890, IV., p. 35) to taking up, point by point, the objections raised by Baumgarten and his students, and according to his thought succeeded in answering them. In the latter communication he makes the remark (p. 84) that he has often been accused of claiming for phagocytosis the entire influence in the production of immunity, denying any other as assisting the organism to disembarrass itself of the bacteria. He repeats again that the part of the phagocytes is a very important one in the production of immunity in general (and of anthrax in pigeons especially), but this does not in any

wise prove that some other influence will not some day be found assisting the phagocytes in their action — an influence that has as yet escaped our observation.

Ziegler also, from whose text-book Metchnikoff had received his first stimulation, took up a strong position against the theory, considering that the intervention of these cells was purely fortuitous, and that the part played by them in infectious diseases was entirely accidental as against the action of the bacteria. His students and assistants after experimenting upon the subject reached the specific conclusion that phagocytosis had nothing to do with the phenomena of immunity to be found in anthrax and symptomatic anthrax, as supposed by Metchnikoff; but this work was also carefully reviewed by independent observers (Lubarsch, Ruffer, Leclainche, and Vallais) and by Metchnikoff himself, and the correctness of the observations was again demonstrated, by which the assertions were supported that phagocytosis was an active force in these diseases.

Other pathologists — notably Virchow (Virch. Arch., 1885, T. CI., p. 12), Ribbert (Deut. Med.

Woch., 1890, no. 31, p. 690), and Hess (Virch. Arch., 1887, T. CIX., p. 365) were more inclined to favor the theory, and Ribbert noted especially that in the reactions produced by the staphylococci there was a modification of the phagocytic action manifested by the collection of the leucocytes about the bacteria — this collection acting as a hurtful surrounding to the bacterium; although Metchnikoff considers this preliminary gathering as simply a first stage to the following englobing and digestion of the bacterial cells by the leucocytes.

Some theories in opposition to the suggestions of Metchnikoff were offered by the bacteriologists interested in the subject, and according to Metchnikoff the starting point of these theories was the observation made by Fodor (Deut. Med. Woch., 1886, p. 617; Arch. f. Hyg., 1886, T. IV., p. 129) that the defibrinated blood of the rabbit was able to destroy a large number of anthrax bacilli. This was the first announcement of the bactericidal property of the blood, and it was at once concluded from it that the body-fluids contained a substance capable of destroying bacteria, and that this property was quite sufficiently

powerful to explain the facts seen in the occurrence of immunity. Nuttall's work (Zeit. f. Hyg., 1888, T. IV., p. 353, in which he refers to Fodor's work as full of errors) was in the same direction and of the first importance. It was based upon observations on the warm stage with the defibrinated blood of various animals, in which it was shown that this same bactericidal property was present — existing, of course, outside of the leucocytes, and that it could be destroyed by subjecting it to fifty-five degrees Centigrade.

Such observations as these formed the first foundation for the humoral theory of immunity, and served as the starting point for innumerable experiments, all of which were intended to show that the theory of phagocytosis had absolutely no foundation in fact.

Behring was one of the first to take up the bactericidal property of the blood as explaining immunity. He (Cent. f. klin. Med., 1888, No. 39) had discovered the remarkable power of the blood of the white rat to destroy anthrax bacilli

with great rapidity — and did not hesitate to conclude that this bactericidal property was responsible for the immunity of the animal to the disease.

To generalize this fact, Behring and Nissen (Zeit. f. Hyg., 1890, T. VIII., p. 41) undertook a long series of experiments. They showed that in animals well immunized against certain bacteria (notably V. Metchnikovii) the blood serum acquired a strong specific bactericidal property, but they also showed at the same time that the blood of animals, even well immunized in other diseases, was very frequently incapable of destroying the bacteria. Therefore, the bactericidal property did not appear to be a general characteristic and was of limited importance; and such facts led Behring to abandon the idea that the bactericidal property was an active factor of a general nature, and essential in the production of immunity.

Buchner confirmed Nuttall's assertion that the bactericidal property disappeared at 55° C.; demonstrated the part that the salts play in the exercise of the bactericidal action; and

especially insisted upon the fact that this property depended upon the presence of special substances of an albuminoid nature to which he gave the name of *alexines*. He also demonstrated the very important fact that the red blood globules of one species of animal introduced into the blood of another would undergo destruction (a globulicidal action) similar in nature to the bactericidal action already demonstrated.

In France, Bouchard especially adopted the humoral theory as explaining immunity (Les Microbes Pathogènes, Paris, 1892), and was strongly supported by Charrin and Roger, his students. Most of their work was done with the bacillus pyocyaneus and their results confirmed them in the belief that the condition of immunity was due to the action of the body fluids, and not in any case to the action of the cells. They attributed both natural and acquired immunity to a special property of the body fluids, and considered that the part of the phagocytes was secondary, that they were concerned simply in carrying off the bodies of the

bacteria, either dead or rendered inoffensive by the previous action of the body fluids.

"The humoral theory made many converts in all parts of the world, and was and is very generally accepted. It has, however, been queried by a few observers whether the phenomena seen to take place in the test-tube really are the same as those that occur in the living body tissues, and it has been shown that similarity of action does not uniformly exist. It has been shown many times that the blood of animals susceptible to infection is bactericidal to the specific bacteria of that infection; while on the other hand, the blood of animals not susceptible to such infection has absolutely no bactericidal properties towards the specific bacterium. Such parodoxes as this are not at all uncommon in the case of infections already investigated, and are so common as to form an almost insurmountable obstacle to a belief in the general action of the body fluids in the production of immunity." (Metchnikoff, *l. c.*)

In reference to what has just been said, as long ago as 1889 (Cent. f. Bakt., 1889, T. VI., pp. 481, 529) Lubarsch carried on a long series

of experiments, in which he showed that animals may be very susceptible to a very small number of bacteria, whose blood-serum is exceedingly bactericidal to a much larger number of the same bacteria in the test-tube. For example, the defibrinated blood and the blood serum of the rabbit destroys numbers of the anthrax bacilli, whilst the animals themselves succumb also very promptly to the injection of a small number of the bacteria in the blood vessels. The assertion is made that such a contradiction in results is only to be explained by the fact that the blood itself undergoes a profound change *after its removal from the body.*

In 1891 the discovery by Behring and Kitasato of the existence of the antitoxines had not made much impression so far as being considered a factor in the production of immunity was concerned. It had been demonstrated as occurring in the two diseases of diphtheria and tetanus, and by Ehrlich for the vegetable alkaloids — ricine, robine, and abrine; but it was thought of as a special phenomenon peculiar to these few diseases rather than as a general principle capable of wide application. It was after

the International Congress of that year that Behring attempted to argue that antitoxine formation occurred in all forms of acquired immunity, and that bacteria introduced into the bodies of animals containing this principle were incapable of producing any hurtful results.

Taking up this point, Metchnikoff undertook to investigate the acquired immunity occurring in pneumo-enteritis of hogs (hog-cholera?). He demonstrated (Ann. de l'Inst. Pasteur, 1892, T. VI., p. 289) that the resistance of the animal to the bacterium in this case does not depend upon the formation of an antitoxine — which is entirely wanting in this form of acquired immunity. (He demonstrated this in the paper spoken of, as follows: he first tested the serum of animals immunized to pneumo-enteritis, and found that it made a favorable medium for the cultivation of the bacillus; there was, therefore, no bactericidal action in the serum. He injected mixtures of the serum of animals dead of pneumo-enteritis (hog-cholera?) with the serum of immune animals and with the serum of non-immune animals, and in both cases found that

these mixtures produced just as fatal results as when the serum of animals dead of the disease was used alone; there was therefore no antitoxine present.) In this same article he also established the fact that the serum of immune rabbits possessed a very active preventive power against infection with the bacillus of pneumo-enteritis (hog-cholera?), which he lays stress upon as being the first time that an *anti-infectious* property had been demonstrated, in addition to the bactericidal and antitoxic properties of the serum, this anti-infectious property being, in accordance with his explanation, a stimulation of the phagocytes in their struggles against the bacteria. The same sort of reaction has since been shown to occur in typhoid fever and cholera. Richet and Héricourt (C. Rend. de l'Acad. des Sciences, 1888, T. CVII., pp. 690, 748) had demonstrated before this an immunizing action in the serum of animals resisting inoculation with the staphylococci, and when Behring and Kitasato discovered the presence of the antitoxine in diphtheria and tetanus, it was supposed that this staphylococcus immunity was also antitoxic in nature. It was shown to be of the same kind

as that in pneumo-enteritis, however — of the anti-infectious nature, and that the serum of the immune animals did not contain an antitoxine. As just stated and referred to by Metchnikoff, the same thing was shown to occur in the artificial disease produced by the injection of the cholera spirilla; in Pfeiffer's results (Zeit. f. Hyg., 1894, T. XVI., p. 268) in cholera he obtained a serum from highly immunized animals with no antitoxic power, but with very active anti-infectious properties.

Such instances as these, together with many others that may be found admirably summarized in Metchnikoff's book upon Immunity (chapter VI.), together with the demonstration of a marked excitation of the phagocytic reaction, served at that time to turn the scale of judgment in favor of the cellular theory of immunity, but the discovery of "Pfeiffer's phenomenon" seemed at the time to upset it entirely just as it was about to be "at least admitted into court." Buchner in the meantime had attempted to reconcile the two theories by supposing that his "alexines" originated in the phagocytes, but that they were present in the body fluids and

performed their functions there, while the englobing and digestive action of the phagocytes was a secondary process.

Pfeiffer's phenomenon seemed to show, however, that there was no cell action whatever necessary in the production of immunity, for it took place in a fluid apparently entirely free from cells. It consisted (Zeit. f. Hyg., 1894, T. XVII., pp. 1, 355) in the following: In working to secure immunity in guinea-pigs against experimental cholera he found that living and virulent cholera spirilla injected into the peritoneal cavity of a fresh guinea-pig together with a small amount of the serum of an immunized guinea-pig, and then examined at short intervals, became bunched together, granular, and finally disappeared entirely. This destruction he claimed to depend entirely upon the action of the body fluids, and to be the result of the action of a substance wholly different from the alexines of Buchner; the immune serum in an inactive state did not contain it by itself, but as soon as it was introduced into the tissues of a fresh animal, the bactericidal substance undergoes some change as a

result of "the activity of the endothelial cells," changes to an active condition, and becomes capable of destroying great numbers of the spirilla. This activity of the endothelial cells was developed into a "new fundamental law" of immunity (Deut. Med. Woch., 1896, pp. 97, 119), and the discovery renewed the vitality of the "humoral theory."

As Metchnikoff (*l. c.*) himself goes on to say, it may be easily supposed that he undertook the investigation of this phenomenon of extracellular destruction of the bacteria as soon as possible, with a view of determining its real importance in the question of immunity. He was soon able to show that Pfeiffer's phenomenon takes place in special circumstances only; previously existing phagocytes must undergo much damage before the spirilla can become transformed into granules; such "phagolysis" is indispensable in order that Pfeiffer's phenomenon may occur in the peritoneal fluids. If it be suppressed by preparing the phagocytes beforehand — by the injection of various liquids — an instantaneous phagocytosis is produced instead of

Pfeiffer's phenomenon; in places where there are no or almost no preëxisting leucocytes, Pfeiffer's phenomenon does not occur at all. The same with the cholera vibrio, — the extra-cellular destruction does not exist except under special conditions, and the vast majority of other bacteria do not show this phenomenon at all under the conditions in which the cholera vibrio manifest it.

Metchnikoff interprets the facts as indicating that this destruction of the bacteria takes place in the tissues as the result of the action of the soluble ferments of the phagocytic digestion. These ferments are normally found in the interior of the phagocytes, and not outside except as the result of the destruction or temporary injury of these cells.

Such a conclusion was in direct contradiction to Pfeiffer's belief, who laid especial stress upon an action of the endothelial secretions. So that, to prove that these latter had nothing to do with it, the reaction should occur outside of the body. This Metchnikoff easily did by adding a little lymph, full of leucocytes, to an inactive anti-in-

fectious serum — when the transformation of the cholera spirilla into granules promptly made its appearance.

This result was what led Bordet (Ann. de l'Inst. Pasteur, 1895, T. IX, p. 462, and 1896, T. X., p. 760) to study the intimate nature of the reaction. He was successful in producing it, not only by adding a little of the peritoneal fluid to the specific serum, but also by adding a little of the fresh blood from the same animal (from which the peritoneal fluid had come). These and other results of his work brought him (Bordet) to the generalization that the destruction of the bacteria in immunized animals results from the action of *two substances*, — one of these is the alexine of Buchner, which under normal conditions is to be found in the leucocytes. It produces bacteriolysis properly so-called when it exists normally within the cells, and also when it has escaped from them as the result of phagolysis. There must, however, be present a second substance in addition to the alexine, and this is the "substance sensibilisatrice" of Bordet, which circulates in the plasma

and carries the "specific" property that is not possessed by the alexine at all.

Bordet continued his most important studies (Ann. de l'Inst. Past., 1898, T. XII., p. 688; 1899, T. XIII., p. 273) by attempting to determine the fate of the red blood corpuscles in the animal body as the result of hemolysis. He established the exceedingly narrow line between the development of the bacteriolytic and the hemolytic properties of the serum of animals, prepared by repeated injections of bacteria and of blood, and his results were soon confirmed by Ehrlich and Morgenroth (Berl. Klin. Woch., 1899, p. 6), who added the important conception that the sensitizing substance of Bordet (the intermediary body of Ehrlich and Morgenroth) has the property of fixing itself in the red corpuscles. The work of these three observers during the last few years has rendered it possible to get some conception of the mechanism of the action of the two substances on the bacteria and the cells of the animal tissues, as will be more clearly stated below.

Metchnikoff (*l. c.*) summarizes the present status of affairs in substance, as follows: The theory of Ehrlich is not in opposition to the theory of phagocytosis. Ehrlich considers that the bacteriolytic ferments as well as the cytotoxic-alexines or complements (called cytases by Metchnikoff) exist in a soluble condition in the circulating blood plasma, whilst Metchnikoff believes that they are normally present in the interior of the phagocytes. This has nothing to do with the Ehrlich theory in regard to the receptors or lateral chains, which considers the antitoxines and certain other anti-bodies (intermediary substances) as products detached from the cells and having an affinity for the toxines and bacterial products.

The theory of phagocytosis seeks to establish the part played by the cells in the destruction of the bacteria. It premises that the vital phenomena of the phagocytes, such as motility, sensitiveness (chemiotaxis), and voracity are essential conditions for relieving the tissues from the bacteria, for the true bacterial ferment is contained within the phagocytes except when phagolysis has occurred. The destruction of

bacteria follows the laws of the destruction of formed elements in general, and this resorption is, in the last analysis, the work of two soluble digestive ferments, of which one (fixateur, immune body) is easily excreted by the phagocyte into the plasma of the blood and exudations, and the other may exist there at all times.

The theory of the phagocytes attempts to establish these principles in as exact a fashion as possible, but it has not attempted to penetrate the depths of the phenomena of intracellular digestion, which is mixed up with the action of soluble ferments in general, and this problem is still far from being solved.

In spite of the numerous objections, the theory of phagocytosis within the limits indicated has not only not been overthrown, but appears to have slowly and gradually gained strength, as is shown by the marked disappearance of opposition that is very noticeable of recent years. Such changes are shown by the instance given (Metchnikoff, *l. c.*) of Buchner's change of attitude toward the theory (Muench. Med. Woch., 1900, p. 1133). At the International Medical Congress in Paris he maintained his theory of

the leucocytic secretion of the alexines, but he went a step further than had before been the case; he admitted that phagocytic activity has a decisive importance in many cases in overcoming the infectious processes, particularly in those in which the alexines secreted have been unable to produce more than a passing diminution in the vitality of the bacteria. In such conditions the bacteria have been so modified that their chemical functions have been transformed into a latent condition from which they would be able to revive in full vital energy if the phagocytes were not present to prevent them from doing so. In any case, as Metchnikoff says, this conception is far from Buchner's original theory, according to which the phagocytes are to be considered only as capable of englobing dead or harmless bacteria. He further speaks of other marked changes in opinion which have occurred, and sums up the present condition of things as follows: the theory of phagocytosis has been strengthened by the demonstration of the following facts, that the phagocytes, in cases of immunity, englobe and destroy living and virulent bacteria, without these having been previously

deprived of their toxines; that the phagocytes contain the bactericidal cytases and produce the fixatives; that the phagocytes absorb the toxic substances. The great contradiction between the occurrence of the bactericidal property and the non-occurrence of immunity is to be explained by the fact that the bactericidal substances in the living tissues remain in the leucocytes and do not escape except when the cells have received some injury. The fact brought out by Gengou, that the blood plasma has no bactericidal property, has given the "coup de grace" to the bactericidal theory of the body fluids.

This work of Gengou (Ann. de l'Inst. Past., 1901, T. XV., p. 231) is one of the strongest supports of Metchnikoff's theory. In it he attempted to determine whether the properties of the shed blood are actually the same as those of the blood in the body. The general results were of great importance in showing that in blood received under conditions preventing the appearance of coagulation there would appear no, or almost no, bactericidal properties, while

in the same blood after coagulation there would appear marked bactericidal powers.

If further experiments serve to support these, and to show that under ordinary circumstances there is no bactericidal property in the circulating blood, it can easily be seen how important this would be for Metchnikoff's theory of the formation of such a property in the cells as the result of vital action.

Metchnikoff goes on, in his summing up, to say that; There is only one constant element in immunity, — either natural or acquired, — and that is the property of phagocytosis. The extent and importance of this factor cannot be denied. It has been fully shown that the phagocytes are sensitive cells that react against morbific agents whether they are organized or not. These cells englobe the bacteria and absorb the soluble substances. They seize upon bacteria that are living and able to exercise their hurtful properties, and subject them to the action of their cellular contents which are able to kill and digest the bacteria, or at least prevent their

pathogenic action. The phagocytes act by virtue of their vital properties and the power of exerting a fermentative action on the morbific agents. . . .

This is a brief statement of the position of Metchnikoff's theory of Immunity, by which the facts seen are supposed to be due in large part to the vital activity of the mesodermic cells, which have retained their power of englobing and digesting foreign substances. The full details can of course only be obtained by a study of his book upon Immunity, in which may be found a most complete summary of the work done, with the interpretation of the results from his point of view.

III.

We come now to a discussion of Ehrlich's theories in explanation of the same phenomena, which are based upon the idea that the reactions seen are primarily chemical in nature, although, as will appear, it is impossible to neglect the vital factor even in these theories.

Dr. James Ritchie (Review of current theories regarding immunity. The Journal of Hygiene, 1902, Vol. II., nos. 2, 3, 4) has published a very elaborate and painstaking discussion of these theories, the best and most complete that has yet appeared in English. It is, however, useful only for those who already have some familiarity with the subject.

It has been attempted, therefore, in what follows to condense the material gathered, to the end that confusing details may not enter, and that merely the main facts may be brought out. The method adopted for illustrating the reactions supposed to occur is entirely original, and

is evidently capable of indefinite expansion, as well as of being applied to all the reactions at present known to occur.

Ehrlich first conceived his theory, or at least the beginning of it, to explain the occurrences in passive immunity; specifically, those seen in the application of the antitoxine treatment of diphtheria.

For the proper understanding of the case the two types of bacterial action must be borne in mind. The first, of which diphtheria is an example, is that in which the results seen follow the action of soluble poisons; which are developed during the growth of the bacteria; which pass away from the site in which the development of the bacteria may be going on; and which produce their results at a distance from that site. Diphtheria and tetanus are both examples of this form of bacterial action; in both of these diseases the bacteria producing them grow mainly in one place in the body, and produce poisonous substances that act at a distance from this place. In the cases mentioned, the poisonous substances also act upon special

groups of cells. Therefore it happens that the toxic substances are brought into existence outside of the cells, or at any rate can be separated from the bacterial cells with ease, and may go on producing their results after the bacteria themselves have disappeared. In cultures, also, the filtrates are found to contain the poisonous products, whose action can be studied in such filtrates, whilst the residue, consisting of the bodies of the bacteria, is found to have very little or no poisonous action whatever. The actual constitution of these poisonous products is not known, in spite of all the work done upon them, but they are examples of *extra-cellular* toxines, and they have the common property of being precipitated by agents that also precipitate the intermediate products of ordinary digestion— the albumoses, — a fact that is of interest, for it is possible that these toxic bodies may not be far removed in composition from those that under normal conditions form the food of certain of the tissue cells.

Bacteria which grow in this way more or less locally and produce their effects most promi-

nently by means of "soluble" toxines are members of the first group.

The second group of bacterial diseases, however, is a most important one, and in it there is seen no such production of separate and soluble toxines. In the bacteria concerned, the filtrates of bouillon cultures have little — or absolutely no — poisonous properties, and the effects in the diseases produced by them are more or less directly connected with the bacterial cell itself. These diseases are the result of the growth of bacteria, which if they act by the production of toxines at all do so by what are called "intracellular toxines." These substances are intimately connected with the bacterial protoplasm and cannot be separated from it, or if they are, only with very great difficulty. Pneumonia, the septicemias, typhoid fever, cholera, plague, and tuberculosis (as a chronic disease) are examples of this form of bacterial action. The general characteristics of all diseases of this type are fever as a general manifestation, and of inflammation as a sign of the local presence of the bacteria. In the bacteria producing results of this general

character, bouillon cultures, filtered, show in the filtrate very slight toxic power, and the bodies of the bacteria left behind often give evidence of containing much more powerful substances.

An illustration of the conditions resulting from such properties is furnished by the many curious facts developed in attempting to secure protection against cholera and typhoid. In both cases it is possible to immunize an animal by the use of small and gradually increasing doses of a virulent culture of the typhoid bacillus, or cholera spirillum, but the serum of an animal so immunized is found to have no power of protecting another animal against injections of the toxines, but only against injections of the bacterium itself — representing, of course, Metchnikoff's "anti-infectious" condition. The same thing is true in regard to plague.

The two groups of diseases outlined above should be carefully distinguished from each other, for there is a marked difference in the practical results that can be obtained in each — in the degree (at present), in the method, and in the explanations offered, in accordance with

Ehrlich's theories, of the reactions by which they are obtained.

In the first group — those in which soluble poisons are formed — the results are secured by successful efforts to protect the tissues against the soluble toxines, without reference to the presence or absence of the bacilli themselves. In the second the actual bacterial cells themselves must be considered as well as the toxic substances that are very intimately connected with them and only to be separated with difficulty.

Ehrlich's theories were first elaborated to explain the conditions seen in the protection of the tissues against the antitoxine of diphtheria (passive immunity), and were based upon the fundamental supposition that the action of the antitoxine upon the toxine was of a chemical nature; and this opinion was supported, among other facts, by the demonstration that the two can be titrated against each other "like an acid and an alkali, and that it takes place more readily with concentrated solutions than with weak." The

experiments with porcelain filters coated with gelatine were also brought forward to show that the reaction is an actual chemical combination. In them it was found that at first, in mixtures of toxine and antitoxine, the latter would not pass through, but the former would; later, however, a neutral fluid would appear, which was interpreted as showing that an actual chemical combination had taken place.

Subsequent observations showed that a simple chemical reaction was not sufficient to explain all the conditions. It was found that toxines lost a considerable degree, if not all of their power, if allowed to stand for some time, an observation that had been made by many observers; but Ehrlich also found that this weakened toxine — to which he gave the name "toxoid" — required the same amount of antitoxine to neutralize it as when fresh. In other words, the toxic power of the toxine had diminished, whilst its combining power remained the same. This being true, the reaction could not be considered the same as a simple chemical neutralization.

The explanation offered by Ehrlich of this loss of toxic power with no loss of combining power is this: he supposes that in the ultimate toxine molecule there exist two chemical affinities (sets of atom-groups), one of which has the power to bind the molecule to a corresponding affinity in the antitoxine molecule, and to which he gives the name of "haptophorous" affinity; but he supposes that there also exists another set of atom-groups, which exert the toxic power of the toxine molecule when the haptophorous group has bound the molecule to another, and this he calls the "toxophorous" affinity or atom-group. In the case of the loss of toxicity observed in toxine that has been kept for some time, he supposes that it is this toxophorous group that has been altered, whilst the haptophorous group has remained unchanged. This supposition, if correct, would account for the condition seen — the loss of toxic property, and the retention of the combining property in as high a degree as it existed at first.

Applying this supposition to actual facts observed, the action of the toxines in diphtheria

and tetanus may be explained in this way; the evidence in favor of a chemical union between the toxine and antitoxine already exists, and in the same way there may be supposed to exist a similar affinity between the toxine and the cells of the tissues upon which it exerts its action. If these affinities be satisfied — if the toxine is bound to the tissue cell by the haptophorous atom-group — the condition called susceptibility appears, and the specific results of the toxic action make their appearance; this of course being the interference with the normal metabolism of the cell by having the cell molecule disturbed and perhaps destroyed.

So, also, an explanation of the production of the anti-toxine may be built up on this supposition. "It is impossible to conceive that the affinities in the brain cell to which, for example, the tetanus toxine becomes attached, are ordinarily of no use in the cellular metabolism. They must bear a part in the latter, or else they would be examples of absolutely useless mechanism in the body. Now the process of immunization consists in its initial stages in the administration

of small non-fatal doses of the pathogenic agent. Looking at the action of the first of these, we see that the cells must be robbed of affinities needed in the ordinary metabolism, by the fact of the attachment of toxine molecules to these affinities or "side-chains." (Ritchie, *l. c.*) The use of the term "side-chain" is unfortunate, for in chemistry it can only be employed in relation to molecules of which the exact composition is known, and this is not the case with any of the toxines; furthermore, it should be employed only in connection with molecules in which the "benzine ring" appears, and of course there is no thought of its presence in these toxine molecules.

Now, in accordance with general biological law, that protoplasm tends to repair injuries if not too great, — and furthermore, that this repair, if it occurs, occurs in excess, — it may be supposed that, when the interference with the cell metabolism is not too great, the tendency is to repair the loss of the haptophorous cell-groups, — which if not replaced would result in the death of the cell; that this replacing goes on to excess, — and that when this occurs the ex-

cess of haptophorous groups are cast off from the cell molecule, and exist free in the blood stream as the antitoxine, ready to combine with such molecules of toxine as may come in its way. When such combination occurs, the haptophorous group of the toxine is taken up by the similar atom-group in the antitoxine. This being so, of course the toxine cannot combine with anything else; if it cannot so combine, its toxophorous atom-group can exert no deleterious influence, and no result is produced; in other words, immunity exists.

Ehrlich has demonstrated the reactions illustrating his theory by the use of figures which are familiar to most persons interested in this subject, but they have been found to be confusing to the beginner. Being figures, and having something the appearance of cells with pseudopodia extending out from their periphery, it has been shown by experience that many students have difficulty in separating the idea of a *vital* action from the reaction which they are intended to illustrate. It has, therefore, seemed best to adopt some other method for illustrating the

reaction just spoken of, and this has been found as follows:

Let H represent the haptophorous atom-group,
T represent the toxophorous atom-group,
and X represent the remainder of the atom-groups in the molecule.

Then the toxine molecule $=H+T+X$,
and the antitoxine molecule $=H+X$.

When two such molecules come together, the haptophorus groups will combine by reason of the affinity they have for each other, and the result is the inability of the toxine to effect further or different combination, so that its toxophorous groups will be unable to exert any hurtful influence: the combination would appear thus : $\begin{cases} H+T+X \\ H+X \end{cases}$

The production of the antitoxine may be illustrated in very much the same way, except that here the reaction lies between the toxine molecules and those having the same haptophorous groups in the tissue cells.

In this case, the toxine molecule is to be represented as before :

$$\text{The toxine molecule} = H+T+X,$$
$$\text{The cell molecule} = H+N+X,$$

in which the N may be supposed to represent the atom-group concerned in nutrition, and X other atom-groups not necessary in the expression of this reaction.

Then the H groups in the two molecules combine (the combination appearing thus:

$$\left\langle \begin{matrix} H+T+X \\ H+N+X \end{matrix} \right)$$

and a double action may be supposed to take place; on the one hand the T group will exert a more or less irritant action on the cell molecule, and on the other hand the H group of the toxine molecule will take up the H group in the cell molecule. Now, under ordinary conditions this H group of the cell molecule is necessary for the proper growth and metabolism of the cell, — if it be taken up for something else, the cell molecule will die or else its H group must be replaced. This replacement occurs in the case we are supposing, and takes place in excess;

the excess of atom-groups is thrown out from the cell and exists free in the blood stream as H+X, which is the antitoxine.

This is not only the simplest way for illustrating what goes on in passive immunity (the use of diphtheria and tetanus antitoxine), but this passive immunity and the production of the antitoxine is the simplest form of immunity that workers in the subject are called upon to explain. In giving the formulæ thus far shown, it is of course to be understood that only the simplest forms are used, and that the X displayed must be supposed to contain all the other atom-groups that are useful in other reactions, but not in the particular one under discussion.

The believers in the physiological explanation of the condition of immunity are not under the necessity for supposing any such reaction as that just illustrated; they believe that the antitoxine excites the cells to resist the toxine, and the evidence for this rests upon the series of experiments (Buchner, Calmette with snake venom, Wassermann) in which it was appar-

ently shown that a mixture of antitoxine and toxine neutral to one species of animal was not neutral to another; if this was so, there could have been no chemical union between the two. These experiments are discredited, however, by the supporters of the chemical theory of union, on the ground that the mixture was not exactly neutral in the first place.

Apparently the strongest support of the theory of chemical union are the experiments of Martin and Cherry (Proc. Royal Soc. of London — quoted by Ritchie, *l. c.*), mentioned above, in which they tested the behavior of mixtures of toxine and antitoxine when passed through a porcelain filter whose pores had been filled with gelatine. The non-appearance of the toxine after a time they interpret as an indication that a complete chemical union had taken place — although the opponents of the theory of chemical action say that the results may be equally dependent upon an actual degeneration of the toxine due to the amount of time elapsing, such as is constantly seen when toxine is allowed to stand by itself.

Another point in favor of the chemical nature of the reaction is brought forward by Ehrlich in the case of the action of the red corpuscles and the alkaloid (?) ricine. By injections of the latter he was able to secure a resistance to it by the red corpuscles of the animal injected, an anti-ricine is formed, apparently of the same nature as the antitoxine in diphtheria, and this serves to protect the red corpuscles against a further action of the ricine. Now, he says, if the union is not of the nature of a chemical reaction, a new physiological function must be ascribed to the red-blood corpuscles, which are not usually looked upon as anything but acting as carriers of oxygen. This supposition, however, goes on the ground that there never will be any new function ascribed to them, which is of course inadmissible.

A great deal of experimental work has been brought forward in support of Ehrlich's theory of a purely chemical relationship between the substances active in the various reactions, and much also that has been interpreted as support-

ing his views, but that is certainly open to other constructions.

The most important seems to be that of Madsen upon tetanus. His experiments were carried on in the test-tube, avowedly to avoid any chance of physiological action, and were of extreme interest and importance. He found that in certain cultures of the tetanus bacillus he was able to demonstrate a substance which he called "tetano-lysin," and which was capable of destroying the red-blood corpuscles, and that this might exist side by side with the ordinary "tetano-spasmin." He further showed, by special methods, that an anti-tetano-spasmin could be produced which would act in a manner precisely similar to that of diphtheria antitoxine.

A most important matter for the support of Ehrlich's theory is the determination of the place where the antitoxine is formed, for if it be supposed that the antitoxine is produced by a specific chemical reaction in the cells, it must also be supposed that this reaction takes place in certain tissue cells and no others. It must be formed in at least some of the fixed cells,

otherwise the experiments of Heymans (quoted by Ehrlich himself) would upset the theory entirely. In these he injected a minimal lethal dose of tetanus toxine and immediately removed all the blood of the animal, at the same time replacing it by the blood of a fresh animal, yet death occurred from tetanus just the same. These experiments as well as many others show that the toxine must act within the fixed cells as well as in the free cells of the blood or body fluids, but just where this reaction takes place has not been definitely proven. The suggestion that the antitoxine may be the result of the splitting up of the toxine molecule, while conceivable, is negatived by the facts thus far observed that seem to show that the toxine molecule is smaller than that of the antitoxine.

Antitoxine is formed in the body somewhere, there can be no doubt of that in the minds of any one who has ever had to do with the immunizing of horses with diphtheria toxine. In these animals, after bleeding and the removal of a considerable proportion of the antitoxine present, there is a return of antitoxic strength in the

blood after a short time, even if no more toxine be injected to induce its return. This has also been shown by Roux and Vaillard in the case of a rabbit immunized against tetanus, in which practically the whole of the blood was removed during a series of days, with no sensible lessening of the antitoxic power; whilst a similar thing was demonstrated by Salomonson in goats immunized against diphtheria. (Ann. de l'Inst. Past., 1893, VII., p. 65; and *Ibid.*, 1898, XII., p. 763.)

All of these instances would seem to indicate that the production of antitoxine is in the nature of a hypertrophy of function, and how great this may be is illustrated in not an extreme degree by Ritchie (*l. c.*, p. 236), in which he demonstrates that an antitoxic serum may be produced one hundred times stronger than the toxine originally introduced; *i.e.*, if tetanus toxine be acted upon by hydrochloric acid until its toxicity be destroyed, it still retains the capacity of giving rise to immunity. By acting for the same time on different portions of the toxine there is no doubt that on each occasion the

state of the modified toxine would be the same. Guinea-pigs were immunized by this modified toxine, and instead of gradually increasing doses, the amount used was always kept the same. One set of animals received four doses of the modified toxine, and another set eight such doses. Of the serum of the first series of animals 0.5 gram was required to protect against a minimal lethal dose of the toxine — whilst of the serum of the second series .005 gram was sufficient to exert the same amount of protection. In other words, twice the amount of toxine gave rise to a serum of one hundred times the strength.

Of course many objections have been raised against Ehrlich's theory, and after all is said there are many points that are still obscure. These objections are nowheres brought together as in the papers by Ritchie (*l. c.*), and the first is this: How is it that after an animal has been brought to a very high degree of immunity, consisting according to Ehrlich's theory in the presence of a large number of antitoxine units in the blood, free and ready for combination,

that the toxine is not neutralized by these free antitoxine units before it gets a chance to reach the cells where its function will be to excite the production of more antitoxine units? Even after it gets to the cells, why is it not taken out of them by the stronger affinities of the haptophorous groups existing in the blood-stream, as is known to be the case in ordinary diphtheria immunity? As a matter of fact the toxine is not destroyed in this way, and upon injection it does go on increasing the activity of the cells in throwing off fresh haptophorous atom-groups, and this is a difficulty that is not easy to explain away. This objection does not arise from the physiological point of view, for the first irritation of the cells is a continuous one and does not require the supposition of the existence of free antitoxine molecules in the blood stream.

The only possible explanation in accordance with Ehrlich's theory is that the affinities in the tissue cell are a little stronger for the toxine than are the affinities in the antitoxine; so that when the combination of toxine and antitoxine reaches the tissue cell, as it must in circulating

through the blood, the H group of the tissue cells overcomes the attraction of the H group in the antitoxine, and binds the toxine to itself, so that the irritant action of the toxine may occur. This, however, is no explanation, for it supposes exactly the opposite of what was supposed in the previous clause: in the present instance, that the H groups of the tissue cells have a stronger affinity for the H groups in the toxine than have the H groups of the antitoxine, while in the preceding paragraph the opposite supposition is made to explain what is seen in diphtheria immunity.

Another very perplexing question is brought forward in the same place: What is it that produces the safety of the animal in the very early days of the process of immunity against a toxine? Active immunity? In experiments quoted by Ritchie (*l. c.*), and upon tetanus toxine, it was shown that 0.5 gram of antitoxic serum was sufficient to neutralize one minimal lethal dose of the tetanus toxine; as a matter of fact, the animals resisted in the series spoken of an average of one hundred and ten minimal lethal

doses (if the same proportion of serum containing antitoxine were required as just stated this would need fifty-five cubic centimeters of serum, and an average guinea-pig does not hold so much blood); but where is it possible for the extra antitoxine units to come from? The conclusion is forced upon us that resistance to a toxine is not necessarily related to the possession of antitoxic power in the serum, but if we are forced to this conclusion, what becomes of the whole theory? In the experiments spoken of above (Ritchie, *l. c.*), the animals could certainly resist sixty-six minimal lethal doses without the slightest symptoms of tetanus, and no part of Ehrlich's theory is as yet capable of giving an explanation of how the toxophorous groups of this amount of toxine are prevented from acting.

Further, in the later stages of Active Immunity — to secure a "strong antitoxine" — thousands of minimal lethal doses are injected. In accordance with Ehrlich's theory, the toxine must combine with the tissue cell in order to stimulate the cell to the production of fresh atom groups (H groups, which shall exist in the

blood as antitoxine). If this process goes on, the T groups may produce their effects, but why do not the thousands of extra T groups produce some result, when it is known that in the beginning *one* minimal lethal dose will do so?

Many other objections and difficulties are brought forward against the theory, which of course cannot be expected to explain all the phenomena in such obscure reactions as these of which we are speaking, but perhaps the one that, whether valid or not, requires more accurate terminology in those speaking upon the subject, is brought out by the use of the formulæ above displayed. The expression is constantly heard that these atom-groups (receptors, side chains, radicals) are produced in the cell in excess, and thrown off to exist free in the blood as the antitoxine or other antibody, as the case may be. If the chemical reaction can be expressed at all by such a formula as employed above, what is thrown off is a radical — an incomplete substance — and *cannot* exist free in the blood as would be supposed from the expressions used in regard to it. It is therefore necessary to

suppose that the radical combines at once with something else — which I have represented by X — so that the unfortunate conclusion may not be forced upon us, that a skilled chemist has put forth the supposition of the existence of something in violation of the laws of all known chemistry.

In considering difficulties like these one is reminded of the remark of Metchnikoff, — spoken of earlier, — that he does not seek to penetrate the laws governing intra-cellular digestion, which has to do with processes as yet absolutely unfamiliar to us.

A way out of the difficulties presented has been found by the suggestion that there is an essential difference between the two forms of immunity — active and passive; that the first (active) has to do with the cell, and should be called Isopathic, and that the second has to do with the fluids (principally the blood) and is the ordinary antitoxic immunity.

It was said in beginning to speak of the subject of Immunity in the case of those diseases in which the results are due to the production of

soluble toxines, that it was the simplest part of the problem. It will, however, by this time appear that the term "simple" is of relative significance only.

The second group of diseases produced by bacteria attracted attention very soon after the facts in regard to the production of passive immunity in diphtheria and tetanus were known.

It was at first supposed that similar antitoxines could be secured in these diseases also, — such as typhoid fever, cholera, and plague, — but it was quickly demonstrated that this was not the case, except to the very slightest extent. In these diseases, and others in which the bacteria do not produce soluble poisons, the actual presence of the bacteria is necessary for the results to appear, and it soon became evident that the production of immunity in such processes must be dependent to a great degree upon the destruction of the bacteria themselves rather than to a neutralization of any poisons that they might elaborate.

Both active and passive immunity can be secured in such diseases, but the latter only to a very slight degree.

The active immunity secured is obtained by the injection of non-fatal or attenuated cultures of the bacteria — or by the use of virulent cultures that have been killed by heat (as in the procedure of Wright and Semple against typhoid fever).

The passive immunity is of the slightest degree, and the curious fact is developed that in both typhoid and cholera the serum of animals treated with dead cultures of the bacteria of these diseases is bacteriolytic to the living and virulent bacilli, but not antitoxic to the toxine.

Bordet's publication of his results in the study of hemolysis, and the light they threw upon Pfeiffer's phenomenon, were the first steps towards the explanation of the phenomena seen in the second group of bacterial infectious processes. Pfeiffer's phenomenon is one of a purely bacteriolytic nature (of course bactericidal also, although a bactericidal serum need not be bacteriolytic), and one of the ways in which it can

be secured is by adding the serum of an animal immunized to the cholera vibrio, and that has also been subjected to a temperature of 55° Centigrade for half an hour, to a culture of active and virulent cholera vibrios, and injecting the mixture into the peritoneal cavity of a fresh animal. The bacteria are soon seen to become gathered together, granular, and gradually to disappear. The action is specific and limited to animals immunized to the disease produced by the bacterium employed — in the case cited, of course, the cholera vibrio. It was at first supposed that a similar reaction could be demonstrated for other bacteria, and that such a reaction could be utilized for differentiating species and races of bacteria from each other.

The significance of this reaction was not understood until the publication of Bordet's results in hemolysis above referred to. In these he found that upon injecting the blood corpuscles of one species of animal into the blood stream of another, there resulted in this blood stream the appearance of a substance that was able to destroy the blood corpuscles of the first species of

animal — a hemolytic or globulicidal property was developed. A specific example is that of using the blood corpuscles of a rabbit for injection into a goat; in a short time there appears in the goat's blood an antibody to the rabbit's corpuscles, which will destroy those corpuscles whenever it is brought in contact with them. This hemolytic property, it was also found, would disappear upon heating the serum containing it for one-half an hour to fifty-five degrees Centigrade. The very remarkable fact was, however, brought out that it would reappear upon the addition to the heated serum of a little fresh serum, although this fresh serum itself possessed no hemolytic property.

These are similar to the occurrences going on in Pfeiffer's phenomenon (of bacteriolysis), and with others formed the basis of an extension of Ehrlich's theory to explain what is seen to occur in immunity against infection.

In explanation of the facts seen in hemolysis, Bordet, and Ehrlich as well, suppose that there must be two bodies present to permit the completion of the reaction. One of these exists in

normal serum, and may be destroyed by heating to 55° C. for half an hour; its presence is demonstrated by such an occurrence as that above mentioned, in which the hemolytic power returns after the addition of a little *fresh* serum, not of itself possessing the lytic property. This substance (in the fresh serum) is called "complement" by Ehrlich (also "addiment"), and is the alexine of Bordet, Metchnikoff, and other writers; it is probably also the same as the "alexine" of Buchner, although there seem to be some minor differences in the thought in regard to their identity.

The second substance exists in the serum of an animal subjected to the process of immunization, and can be destroyed by being subjected to heat at 75° C., for one-half hour. To this substance Ehrlich has given the name "immune body." It corresponds as nearly as may be to the "substance sensibilisatrice" of French writers and supporters of the cellular theory of immunity.

In order to develop his theory of chemical action in explanation of the condition of immu-

nity in this group of diseases, Ehrlich supposed that in this immune body there exist two haptophorous atom-groups of affinities — not one as before — and that one of them was concerned in satisfying a similar haptophorous atom-group (receptor) in the blood corpuscle (of the same nature as the receptor in the tissue cells in antitoxine immunity); and the second haptophorous atom-group of affinities (receptor) is satisfied by a similar group in the complement, which, as has been seen, exists in the serum (blood stream?) of fresh animals.

In the complement there is supposed to exist a group of affinities analogous to the toxophorous group of the toxines — except that now it is supposed to exist normally in the blood stream (or cells of the body, according to Metchnikoff), and this atom-group acts as the hemolytic or bacteriolytic, or, in general, the cytolytic substance.

The experimental evidence upon which this theory rests is as follows: If a goat be treated with repeated doses of sheep's blood, there develops in its serum the capacity of dissolving

sheep's red-blood corpuscles. (It may be said that a great number of similar hemolytic sera can be obtained by treating one species of animal with the blood of another species.) Ehrlich took four cubic centimeters of five per cent defibrinated sheep's blood in .75 per cent salt solution, added one cubic centimeter of immune goat's serum which had been heated half an hour to 55° C. (and which thus contained only immune body), and placed the mixture for fifteen minutes at 40° C. The question of where the immune body was, he now investigated in the following way: the mixture was centrifugalized until all the corpuscles were deposited at the bottom of the tube; the supernatant clear fluid was decanted, and there was added to it .2 cubic centimeter of ordinary sheep's blood (containing, therefore, susceptible red-blood corpuscles) and .8 cubic centimeter of "fresh" goat's serum (containing, therefore, goat's complement); the mixture was placed at 37° C. for two hours without any trace of hemolysis occurring. Now, if the immune body had been left in the fluid after centrifugalizing, the complement from the fresh goat's serum ought by it to have been

linked to the sheep's corpuscles, and hemolysis of the latter ought to have occurred. The immune body therefore was not here. The sheep's corpuscles of the original mixture were, of course, in the deposit separated by the centrifugalization. This was now taken, stirred up with four cubic centimeters of .75 per cent salt solution and there was added .8 c. c. of fresh goat's serum (containing of course complement). The mixture was placed at 37° C. for two hours, and at the end of this time there was found to have occurred hemolysis of the corpuscles. During the fifteen minutes that the original mixture was kept at 40°, therefore, the immune body in the immune goat's serum had united itself to the sheep's red corpuscles, as was evidenced by the fact that when the latter were exposed to fresh complement, hemolysis occurred. As remarked above, complement cannot cause hemolysis by itself. The method of the experiment is that three factors are necessary to the occurrence of a given hemolysis: the red-blood cells to be acted on; a body resistant to heat, occurring in the serum of the immune animal (immune body); and a body susceptible to

heat, occurring in the serum of an unimmunized animal (complement). When the presence of any one of these substances is suspected, it can be traced by adding the other two, and observing whether hemolysis takes place. In the investigation, sheep's blood was taken next, fresh goat's serum was added, the mixture centrifugalized, and the fluid on the one hand and the deposit on the other investigated for complement. None was found in the deposit, but it was found in the clear fluid, so that no combination had taken place between it and the blood corpuscles. Next it was found that there was a greater affinity between the immune body and the blood corpuscles than there was between it and the complement. The proof was as follows: It was observed that in a mixture of five cubic centimeters of five per cent sheep's blood, one to one and one-third cubic centimeters of heated immune goat's serum, and one-half cubic centimeter of fresh goat's serum, there was just enough of all the constituents to satisfy all the affinities and leave none over. If all these substances were mixed at 0° C., and the mixture centrifugalized as before, it was found that the

complement present was still free in the supernatant fluid. Therefore the affinity of the immune body for the corpuscles was greater than its affinity for the complement. This last experiment also shows that at 0° C. the immune body and the complement must have existed free, side by side. (Ritchie, *l. c.*, p. 245.)

The reactions that are supposed to take place may be expressed by the following formulæ:

Hemolysis requires for its appearance

1. The blood cell.
2. The immune body (in the serum of the immune animal).
3. The complement (in the serum of the fresh animal).

These may be represented as follows:

1. The blood cell molecule=H+N+X
2. The immune body " =H+*H*+X
3. Complement " =*H*+L+X

The H group of the complement binds the molecule to the immune body by one of its *H* groups, and the second H group in the immune

body binds the two to the blood cells — thus allowing the L atom group of the complement to exert its destructive (lytic) action.

For the production of the hemolytic property (blood immunity it may be called), there are required:

1. The blood cells that are injected.
2. The body cells in the animal into which the above are injected.
3. Complement (present in fresh serum.)

The molecules of each of these and their reaction upon each other may be shown thus:

1. The blood cell molecule=H+N+X
2. The body cell " =H+*H*+N+X
3. Complement, =*H*+L+X

As in the preceding case, the *H* group of the complement is bound to the body cell by the corresponding *H* group, the blood cell by another. The H groups of the body cell are needed for its ordinary metabolism, but are taken up in this way; if the cell is to live they must be reproduced; as the cell does live they are reproduced, and as is usual, this reproduction goes on to ex-

cess; then the H groups not needed are thrown off into the blood stream and exist as

H+*H*+X, or the immune body.

The reactions in hemolysis having been made more clear by the investigations of Ehrlich spoken of above, it became evident that the reaction in bacteriolysis (of which Pfeiffer's phenomenon was the first example) could be explained in the same way — and it can also be shown by a formula similar to that used in the demonstration of hemolysis, as follows:

The bacteriolytic reaction requires for its completion

1. The bacterial cell, molecule=H+T+X
2. The immune body, " =H+*H*+X
3. The complement, " =*H*+L+X

Then, as before, the complement and the immune body are bound together by one set of haptophorous atom groups ; these two are bound to the bacterial cell by another similar set of atom groups, and when this has occurred, the lysin atom group (L) can set up its action and the bacterial cell may be destroyed.

So, also, the production of this bacteriolytic property may be represented as follows :

Its production requires

1. The bacterial cell (which is injected) . . . molecule=H+T+X
2. The tissue cells (from which the immune body is produced) . . . molecule=H+*H*+N+X
3. The complement (present in fresh blood) . . molecule=*H*+L+X

In this reaction the H groups of the body cell molecule are satisfied by the combination with the similar sets of atom groups in the bacterial cell and in the complement ; they are, however, required for the ordinary metabolism of the body cell molecule ; they must, therefore, be reproduced or the body cell molecule will die ; being reproduced, they are in excess of the requirements of the cell — are thrown off and exist in the blood stream as "immune body," ready to combine with fresh bacterial cells as they are introduced, *i.e.*, as

H+*H*+X.

The facts in hemolysis and bacteriolysis have led to the investigation of the action of other kinds of cells when introduced into fresh animals. A very large number of different kinds of action of this nature have been studied, and it is demonstrated that it is possible to secure sera of many sorts which have a specific action upon the cells originally used for the production of the cytolytic power. Thus, for example, the study of the results following the injection of living spermatozoa has been the demonstration of the production, in the serum of the animal in which they are injected, of a property that has the power of immobilizing fresh spermatozoa, if not of absolutely dissolving them ; the same thing holds true of other cells. Liver cells injected in the same way will produce a serum capable of inducing fatty degeneration of the liver cells of a fresh animal. Of course the possible results that may come from such cytolytic action as this are of the most important nature.

The reactions are explained by Ehrlich in the same way as the phenomena of hemolysis and

bacteriolysis, and they may be illustrated by formulæ similar to those already explained.

Thus — cytolysis requires

1. The cell to be acted upon, molecule=H+N+X
2. The immune body (produced by the previous injection of the above) . . . . . molecule=H+*H*+X
3. The complement . . " =*H*+L+X

And when the H groups have produced the necessary combinations, the L atom group of the complement may exert its lytic action.

The production of the cytolytic property may, of course, also be illustrated in the same way as follows :

Its production requires

1. The foreign cell to be injected . . . . molecule=H+N+X
2. The tissue cells in which the injection is made, molecule=H+*H*+N+X
3. The complement . molecule=*H*+L+X.

The same combinations are supposed to occur in this as in preceding cases: the H atom-groups combine together, so that those needed for proper

metabolism of the tissue cells in which the injection is made are used for another purpose — these are reproduced in excess, and cast off into the blood stream, where they exist as substances capable of aiding in the cytolytic action.

Both sides, that which supports the "humoral" and that supporting the "cellular" theory, agree in supposing that two bodies are necessary for the production of these reactions, but there are two special points of dispute — first as to whether the complement exists in the cells or out of them; and second, whether it is single and specific for the race of animals in which it occurs, or whether it is multiple and specific only for the reaction in which it appears active.

There is no dispute as to the complexity or rather multiplicity of the immune bodies, which are generally acknowledged to be very numerous and capable of action only in special directions — on the particular bacteria or tissue cells, as the case may be.

As to the multiplicity of complement, it has been shown that it is possible to reactivate a serum by the addition of a little fresh serum

from an animal of a different species. Such a fact tends to show that complement is the same in all species of animals, although there are other experiments that indicate that this conclusion may not be absolutely warranted.

Still another fact that is of importance is the conclusion warranted by the evidence that complement and immune body are not produced in the same quantity, which would be expected from the circumstances of the case. An illustration is furnished by the possibility of demonstrating that a given quantity of a cytolytic serum may destroy a given quantity of cells. If, however, fresh serum be added, the same quantity of the cytolytic serum will be found capable of dissolving many times the number of cells it could act upon in the first place.

The main point of controversy is upon the multiplicity of complement, but not enough evidence is yet before us to enable us to form a definite opinion upon the matter. Metchnikoff takes very strong ground in the matter, and declares that the complement exists only in the cells of the body, from whence it passes into the

blood stream only after some destructive action on the cells, and furthermore that it may take part in the completion of the immunity occurring in many infectious processes as well as in many cell reactions. On the other hand, Ehrlich argues forcibly for the multiplicity of complement, claiming that this substance is multiple and specific for each reaction, as is the case with immune body.

Walker (Journal of Hygiene, 1902, Vol. II., p. 85) furnishes the specific evidence of the identical nature of complement coming from different animals as follows: he found that the anti-typhoid serum from a horse that has been inactivated by the application of heat — thus destroying its complement — may be reactivated by the addition of serum from the rabbit, ox, and the pig (thus adding fresh complement). This may be represented graphically as follows:

Anti-typhoid serum contains
Complement, molecule =H+L+X
Immune body, molecule=H+*H*+X
Other constituents, molecule=P(roteids)+S(alts)+X.

If this be heated to 55° C., for one-half hour, the complement is destroyed, and the complement molecule (H+L+X) no longer exists. The serum therefore is incapable of exercising its bacteriolytic property. If now the fresh serum of an ox, rabbit, or pig (containing of course fresh complement (H+L+X)) be added, the same constituents as at first would exist in the anti-typhoid serum, and the bacteriolytic power would be restored.

The various facts brought forward show that one of the difficulties that may arise in the treatment of diseases of the second order (like typhoid fever, cholera, and plague) is in the fact that much more immune body may be formed than there is complement to help make active, and it is possible that this may be the explanation of the poor results obtained with antitoxic sera in the diseases just spoken of. Just how this difficulty is to be met has not been shown, but it is true that the apparently simple thing to do, the addition of fresh sera (containing more complement) does not fulfil the requirements, at least so far as experiments have yet gone.

The following summary of this part of the subject is exceedingly good (Ritchie, *l. c.*, p. 261): It is to be observed that the methods by which bacteria are dealt with in the body are similar to those which obtain when many kinds of foreign cells gain an entrance into the latter. The development of artificial immunity against such bacteria depends on the latter being introduced either in a form not strong enough to cause death, or, if virulent, not in sufficient numbers to cause death. In either case, the affected animal probably resists infection because it can develop in its body, or already possesses a substance — immune body — which attaches itself to the bacterial protoplasm, and in virtue of this attachment permits another body, the complement, which exists normally in the animal's body, to act on the bacteria, with a fatal result to the latter. In the case of a further infection with bacteria, such as might occur naturally, or as occurs during the process of immunization, then no illness may result, but a fresh formation of immune body may occur. Whether a fresh formation of complement may occur to any extent is a question for further

investigation, but in an immune serum the complement is always present to a less degree than the immune body. What the nature of these bodies is is unknown, but the complements are less resistant to heat than the immune bodies. Further, the nature of the reaction which takes place between bacteria, immune body, and complement is disputed, and lastly, while the multiplicity of immune body is undoubted it is still open to question whether there is a great number of complements in each animal's body, or whether there is, for each species at least, only one complement which is capable of acting in conjunction with a great variety of immune bodies, so as to produce a solvent effect on many different kinds of bacteria.

One of the greatest difficulties standing in the way of the supporters of Ehrlich's theories is the existence of the bactericidal property in the blood serum of animals that have not been treated in any way, and at the same time are susceptible to the action of the bacterium that their blood serum will destroy. The well-known fact that the blood serum of the rabbit has a

marked bactericidal action upon the anthrax bacillus, whilst the animal itself succumbs very promptly to an infection by these bacilli, is an illustration in point, and is not by any means the only one that can be brought forward.

Whether or not this bacteriolytic action is the same as that seen after the condition of immunity has been produced has not been shown; if it is the same, its production must be accounted for, and the reactions may be explained in the same way as has been adopted for the illustration of the other parts of Ehrlich's theory; if, however, it is not the same, and its presence seems to show that a bactericidal property of the serum does not of necessity indicate a condition of immunity, then the explanation of its appearance and the method by which it acts has not yet been furnished.

Somewhat the same position must be taken in regard to the phenomenon of agglutination. The relation of this phenomenon to immunity does not appear to be any more intimate than that of the possession of a bacteriolytic property on the part of the blood serum. Agglutination,

moreover, seems to be even less connected with any action of a "specific" nature than the bactericidal property spoken of just now. In this connection it may be of interest to repeat some conclusions reached in an investigation upon this matter some time ago: 1, That the agglutinating property does not lie in and is in no way connected with the flagella of the bacteria concerned. 2, That agglutination is not to be accepted as a specific property connected with a condition of immunity, although this is a difficult idea to give up. 3, That the homologous nature of agglutination cannot be considered a positive characteristic, for how then explain the clumping of typhoid bacilli by diphtheria antitoxine? 4, Finally that no universally applicable theory of agglutination has yet been offered. That of Bordet (Ann. de l'Inst. Past., T. XIII., p. 224, 1889) seems to us the most rational yet suggested — that there is an agglutinating agent ("agglutinine") acting upon an agglutinable substance ("substance agglutinée," the nature of which is not yet determined), and that this reaction occurs not only with bacteria, but with many other elements — globules, casein, and

precipitates of various kinds. (Ernst and Robey. Trans. Triennial Congress of Physicians and Surgeons, Vol. V., p. 28, 1900.)

If one favors the idea that phagocytosis is the active factor in disposing of bacteria in the occurrence of immunity from infection, the phenomenon of chemiotaxis must be accounted for, both positive and negative. Metchnikoff does this by the supposition that there exist substances in the immune serum which stimulate the chemiotactic power of the phagocytes toward the invading bacteria. These "stimulines" are supposed by Metchnikoff to exist along with the "cytases," or else to be these bodies themselves, possessing the stimulating property along with the others that have been ascribed to them.

Not enough attention has been paid to the condition of phagocytosis in the phagocyte producing tissues, after the acute stage has passed away, and on this point there is much to be said. Recently the condition of these tissues has been studied by Roger (Les Maladies Infectieuses, Paris, 1902, p. 680 *et seq.*), and Muir (Journal of Pathology, Vol. VII., p. 161, quoted

and summarized by Ritchie, *l. c.*, p. 283 *et seq.*). "The fact has long been known that in many infectious conditions the number of leucocytes in the circulating blood is increased, but these observers were the ones to demonstrate the pronounced germinative activity which occurs in any infection, in the precursors of these cells. With regard to the leucocytic phagocytosis, Muir has shown both experimentally on animals and by observations on man, that in infections where there is a polymorphonuclear leucocytosis, not only is there evidence of an active division of the parent cell in the bone marrow, but so active is this process that the red marrow increases in amount and encroaches on the yellow. In a case of pneumonia, for instance, a few days after the commencement of the disease the red marrow may have increased so as to occupy a seventh part of the whole medullary cavity of the femur. Not only, however, does proliferation occur in the site of formation of such an important class of cells as the polymorphonuclear leucocytes, but Muir has also shown that proliferation occurs during some infections in such fixed cells as those lining the sinuses of

lymphatic glands and also in the hyaline cells lying free in the lymph sinuses, which later may be connected with some at least of the large mononucleate hyaline cells of the blood. He further points out that similar hyaline cells — endothelial cells, connective cells — proliferate during infection, as can be shown by mitotic figures being found. It is no doubt the case that in different infections different groups of cells thus proliferate: in typhoid fever, for instance, there is no polymorphonuclear reaction, but here the proliferation of endothelial cells and hyaline cells in lymphatic glands has been observed. Thus while Metchnikoff has insisted with justice on the importance of the local reaction and of the wandering cells of the body in infections, and has noted the occurrence of phagocytosis in other cells (his fixed ameboid cells), he has missed the fact of the great proliferative changes in various parts of the body which may be described as the reaction of the body generally against infection. It must be insisted upon that there are not only local chemiotactic effects, but in the case of the wandering cells there is the general chemiotactic effect which draws the

polymorphonuclear leucocytes from the marrow, and in all cases of severe infection there is the further stimulative effect which leads cells in various parts to divide. Either this stimulation is part of a reparative process, or it is to be looked on as the result of injury due, say, to circulating poisons. The fact that in relation to one aspect of the process, namely, the polymorphonucleate reaction, the effect is often to increase at a given point the available number of cells capable of playing the part of phagocytes leads one to think that all these tissue changes may be of the nature of an exaggeration of normal functions, the general effect of which exaggeration is to have a beneficial effect. It is to be noted as a very important point in this process that most of the distant effects must be due, even in the case of bacteria which *in vitro* do not produce soluble poisons, to the circulation of soluble toxines, unless — which is possible — we consider bacteria capable of exerting purely physical influences. Connected with these is the other very important fact that embryonic activity may be dissociated from any actual phagocytosis on the part of the proliferating

cells, and this taken along with such facts as the proliferation in certain infections of the non-phagocytic eosinophilic leucocytes raises anew the question of the possible secretion of chemical substances into the serum which may be concerned in the complicated process by which bacteria are destroyed in the animal body. It may also be noted here that Muir has observed an increase in the size and distinctness of the granules in the young polymorphs which occur in the marrow during a severe infection. This might indicate the preparation of material to be secreted. So that from what has just been said it is thus possible that on the fixed cells of the body and the fixed precursors of the wandering cells are impressed qualities which perpetuate immunity in an animal which has survived an infection." (Ritchie, *l. c.*, p. 283.)

Such a conclusion as that just drawn is at least in part in direct accord with Metchnikoff's assertion that there is an inheritance of a tendency towards phagocytosis by the descendants of cells upon which the property has been impressed.

In concluding his review, Ritchie brings forward (p. 452, *et seq.*) the further considerations that follow, and says in regard to them, in substance, that in the first place it is difficult to see how, granted that two substances are necessary for the production of immunity (the immune body and the complement), these substances are to meet the bacterial cell in the first instance except in the body cell. Unless all immune bodies are existent in the serum as "go-betweens" (amboceptors) — substances of an identical nature except that they are utilized for the normal metabolism of the cell until needed for the reactions occurring in immunity — how can the bacterium when it first enters the body come in contact with the immune body for which it has an affinity? The receptor is *in the cell* (as represented in the formula used to illustrate the reaction) and the bacterial cell receptor must also get into the tissue cell — except it be supposed that they exist under normal conditions in the blood stream. It is undoubtedly possible that the immune body may exist in the blood stream as the result of normal metabolism, but this is the only supposition which would explain

how it gets there; if it does not so exist, then the bacterium must be conceded to enter the tissue cell at the first in order to start the excitation resulting in the production of the immune bodies; if this be the explanation, then Metchnikoff's contention that phagocytosis — at least cellular action — takes place in the very first instance must be adopted.

In the second place the query arises as to why, if the bacteria must meet both complement and immune body in the cell, why is the latter produced so much in excess and not the former? On the basis of the supposition that the complement exists normally, and that the immune body is the result of an excess of cell activity set in motion by the taking up of the haptophorous atom-groups needed in the ordinary metabolism by the bacterial atom-groups of the same nature, this is easily understood, because this new vital activity goes on in excess and may be supposed to result in the production of a great excess of the atom-groups representing the immune body.

It is most probably the case that the reactions

occurring in the production of immunity are closely related to those that go on under the ordinary conditions of tissue metabolism, and the only way in which this can be settled is by the determination, in the first place, of what the normal functions of the cells are; and in the second, whether substances similar to the antibodies exist under normal conditions in the body.

Inquiry in the first direction has as yet left much obscurity to be cleared away, and the exact functions of the leucocytes, or of the cells from which they are derived, are not clearly understood. As to the second point, so many investigations have seemed to show the facts that it can hardly be doubted that substances closely allied to if not identical with both complement and immune body do exist in the sera of normal animals — it is, however, disputed as to whether they exist free in the normal blood stream.

If they do so exist they must have some normal function, for it is not to be supposed that they exist for the sole purpose of taking part in the specific reactions of immunity when such necessity may arise. They must take some part in normal tissue metabolism; and in attempt-

ing to determine what these functions were Bordet first called attention to the occurrence of the so-called "precipitines." (Ann. de l' Inst. Past., 1899, T. XIII., p. 225.) He found that in treating rabbits with chicken's blood, a hemolytic serum could be obtained; but also, that when this hemolytic rabbit's serum was added to chicken's blood a *precipitate* made its appearance.

Many such precipitates have been obtained with many kinds of blood, and also with many kinds of bacteria as well. Their nature is not well understood, but the reactions seem to be specific and most exact, and these reactions are of great medico-legal value in the determination of the presence of human blood in suspected stains.

The general reaction may be shown by the same formulæ as were used for the purpose of illustrating the forms of immunity as follows :

The production of the precipitate requires :

1. The blood globule, molecule=H+N+X
2. The immune body, " = H+*H*+X
3. The complement, " = *H*+P+X

The complement and the immune body are bound to the blood corpuscle molecule, and the P atom-group acts in such a way as to produce the precipitate.

The production of the precipitine power requires :

1. Blood serum, molecule=H+N+X
2. The body cell, molecule=H+*II*+N+X
3. The complement, molecule=*II*+P+X

The complement and the blood serum molecule are bound to the tissue cell by the H atom-groups; these latter in the tissue cell molecule are reproduced in excess and are thrown off after this reproduction occurs and exist free as H+*II*+X=the immune body, ready for the production of the precipitine reaction when the need arises.

Any specific reaction — as that resulting from the addition of rabbit's blood to the goat — may be represented by the addition of the sub-letters desired.

The belief that the affinities existing in the body cells are very numerous is forced upon the

supporters of Ehrlich's explanation of the reactions that occur, and this is especially emphasized by the formulæ presented in illustration. It is to be supposed that these affinities are concerned under normal circumstances in helping on the normal food metabolism of the tissue cells, and possibly other reactions. Ehrlich has given the name of "haptine" to the whole group, and that the number is great may be seen by those already known — lysins, agglutinines, precipitines, complements, ferments, anti-complements, ferments, etc.

The production of an anti-complement, for example, may be represented as follows: it may be obtained by injecting serum containing complement from one animal (*e.g.*, a guinea-pig) into another (*e.g.*, rabbit):

The injected serum, molecule=H+L+X

The receiving blood cell, molecule=H+(H+H+H+H+H)+N+X

The two are bound together, the special H group is reproduced in excess, is thrown off, and exists as H+X=the anti-complement.

The action of this anti-complement may be very simply represented :

The anti-complement molecule=H+X
The complement molecule =H+L+X

But the two are bound together, and therefore the L atom-group cannot be bound to anything else, and therefore cannot do any harm or assist in the ordinary action of the complement with the immune body.

IV.

SUMMARY.

A study of the present position of the two main theories explanatory of immunity shows that they are not so far apart as is generally supposed.

Both sides agree that the phenomena seen in active immunity require *two* substances (immune body or substance sensibilisatrice, and complement or alexine).

One side, however, insists that these substances remain in the cells (phagocytes). The other maintains that they exist in the blood stream (although, as has been seen, cell activity is necessary for the production of the immune body, at least). This latter fact of itself prohibits the belief in a purely humoral explanation of immunity.

Both sides agree that "immune body" (substance sensibilisatrice) varies, is multiple and

specific for each reaction in which it occurs; one for typhoid, one for diphtheria, and so on.

They do not yet agree, however, that "complement" (alexine) is multiple. One side maintains that it is the same for all reactions, the other that it differs and is specific for each.

The point that has been insisted upon by Metchnikoff from the beginning is maintained: that in all reactions the cell activity must intervene at some stage of its production.

V.

SUMMARY OF METHOD OF ILLUSTRATING REACTION.

Let H represent the haptophorous atom-group in the molecule.

T represent the toxophorous atom-group in the molecule.

N represent the nutrient atom-group in the molecule.

L represent the lysin atom-group in the molecule.

X represent the remainder of the molecule.

It is not *necessary* to separate the N atom-group; it is done, however, to make clearer the fact that there is some vital action going on.

Then to represent the interaction of toxine and antitoxine the following may be used:

Toxine molecule contains . . . H+T+X

Antitoxine molecule contains . . H+X

The two H groups are bound together, thus:

$$\begin{matrix} \diagup \mathrm{H+T+X} \\ \diagdown \mathrm{H+X} \end{matrix}$$

therefore the T group in the toxine cannot be bound to anything else, and the substance exists as a harmless material in the blood stream.

The production of the antitoxine by the injection of the toxine may be represented as follows:

Tissue cell molecule contains . . H+N+X

Toxine cell molecule contains . . H+T+X

The H groups bind the two together; but the H group of the tissue cell molecule is necessary for the metabolism of the cell; it must be reproduced if the cell is to live; being so reproduced the reproduction occurs in excess, and then the H groups are thrown off and exist free in the blood stream as

H+X, which is the antitoxine.

Bordet's work on Hemolysis, and Ehrlich's later work, showed that two substances were necessary for other reactions, and necessitated the supposition of the existence of *two* haptophorous atom-groups. In accordance with this

idea the reaction in *hemolysis* may be shown thus:

It requires the cell to be destroyed, the immune body, and the complement.

The cell molecule contains . . . . H+N+X

The immune body molecule contains . . . . . H+*H*+X

The complement molecule contains . . . . . *H*+L+X

The complement is bound to the immune body by one H group, the two to the blood cell by the other H group in the immune body, and then the L group in the complement may act. In cases where two H groups occur, the different affinities are indicated by capital and italics.

The production of the immune body requires blood cells injected, the cells of the body in which the injection is made, and the complement. The reaction is shown thus:

The blood cell molecule contains . . . . . H+N+X

The body cell molecule contains . . . . . H+*H*+N+X

The complement molecule contains . . . . . *II*+L+X

The two H groups in the tissue cell molecule are used up, but must be reproduced; being reproduced, this occurs in excess; they are thrown off and exist free in the blood stream as

H+*II*+X, which is the immune body.

So with Bacteriolysis: the formulæ are the same, except in this instance the cell to be acted upon is the bacterial cell, and not the blood cell:

The bacterial cell, molecule contains . . . . . H+T+X

The immune body, molecule contains . . . . . H+*II*+X

The complement, molecule contains . . . . . *II*+L+X

As before the *II* groups in the complement and the immune body combine, and then these two are joined to the bacterial cell molecule by the remaining H groups, and thus the L group in the complement may exert its destructive action upon the bacterium.

The production of the bacteriolytic power requires the bacterial cell to be injected, the tissue cell from which the immune body is to be produced, and the complement existing in the fresh blood stream. The reaction is as follows:

The bacterial cell molecule contains . . . . . H+T+X

The tissue cell molecule contains H+*H*+N+X

The complement molecule contains . . . . . *H*+L+X

The H atom-groups in the tissue cell molecule being taken up, are reproduced, cast off, and exist free in the blood stream as

H+*H*+X, the immune body.

Cytolytic action may also be shown:

The cell molecule to be acted upon contains . . . . . H+N+X

The immune body molecule contains H+*H*+X

The complement molecule contains . *H*+L+X

The H groups are joined together, and the L group of the complement may then act.

The production of the cytolytic property is secured thus:

The foreign cell molecule injected contains . . . H+N+X

The tissue cell molecule, in the body in which injection is made, contains . . . H+*H*+N+X

The complement molecule contains . . . . . *H*+L+X

The H groups in the tissue cells are reproduced in excess, and exist free as H+*H*+X the immune body active in cytolysis.

Precipitine reactions may also be shown:

The blood globulin molecule contains H+N+X

The immune body molecule contains H+*H*+X

The complement molecule contains . *H*+P+X

The H groups being bound together, the L atom-group may act, in this instance producing the precipitate, and therefore it may be more clearly represented as P.

The production of the precipitine power occurs as in all the others:

Blood injected, the molecule contains . . . . . H+N+X

The cell (of the body in which the injection is made) the molecule contains . . . . . H+*H*+N+X

The complement (molecule contains) . . . . . *H*+P+X

The H groups are reproduced in excess as before, and exist free as H+*H*+X, the immune body necessary for this reaction.

Anti-complement reaction is shown :

The complement molecule contains . H+L+X

The anti-complement molecule contains . . . . . H+L

The two H groups combine, and the complement is rendered harmless by this combination.

The production of the anti-complement is shown thus:

The injected serum (containing complement) molecule contains H+N+X

The receiving blood molecule contains H+(H +H+H+H+etc.)+N+X.

The two H atom-groups combine, the one in

the receiving blood is renewed in excess, is thrown off and exists free as

$$H+X,$$ the anti-complement.

It is of course to be understood that the letters and formulæ given above are representative of the general reaction only, in each case. If it be desired to express a specific reaction — as for instance that of the diphtheria antitoxine — it may be done by placing a sub-letter "d" in each place where its need is felt; for example, H_D+X, with the sub-letter at H, represents the special reaction used for illustration in diphtheria; any other reaction may be shown in its specific nature by the same means.

The molecule must also be supposed to contain, in the letter X, all the other atom-groups not needed for the expression of the special reaction under consideration.

[NOTE. — The use of H and *H* to indicate the binding of the complement and the immune body, and of these two to the cell, was suggested almost simultaneously by Prof. Neisser, of Frankfurt, and Dr. Grünbaum, of Liverpool, and had been adopted when their letters came.]

VI.

GLOSSARY.

Active immunity: more or less permanent immunity, the result of the use of gradually increasing doses of the toxines of a bacterium.

Addiment: another term, used by Ehrlich, for complement.

Agglutinines: substances found in the blood (and possibly elsewhere) during and after the progress of an infectious disease, capable of clumping the bacteria producing the infections; non-specific; not dependent upon a condition of immunity.

Alexines: substances existing in the serum of both immune and non-immune animals (perhaps of different nature in each) possessing bactericidal properties. May be free in the blood stream, or always, except by accident, in the leucocytes.

Alexines: (Bordet) bodies recognized by Bordet as existing in the cells and possessed of bactericidal properties; because of the fact that they may be destroyed by heat (55° C.), and thus resembling Buchner's bodies of the same name, Bordet called them alexines; probably the same as the complement of others.

Amboceptors: receptors of the third group, capable of becoming normal factors in metabolism; (Zwischen-körper, go-betweens;) immune bodies.

Anti-complement: a substance produced in the blood of an animal, injected with serum containing only complement (no immune body); it acts against the complement, and prevents the latter from acting with the immune body.

Anti-immune body: a substance that may be supposed to exist in the blood of an animal treated with immune serum in which only "immune body" exists — the complement having been destroyed.

Anti-infectious: term used by Metchnikoff to

denote the condition of resistance to further injections of the infectious agent.

Anti-sera: the sera of animals which have been treated in such a way as to produce in them substances antagonistic to various bodies; *e.g.*, bacteria, toxines, blood-corpuscles, etc. Most commonly used to denote antitoxic sera.

Antitoxic immunity: passive immunity.

Antitoxine unit: the amount of immune serum necessary to exactly neutralize one hundred minimal lethal doses of toxine, after being mixed one-half hour.

Atom-groups: atoms of the molecule supposed to have special affinities; become receptors.

Autolysin: a substance in serum capable of dissolving the animal's own blood; existence not demonstrated.

Bacteriolysis: the breaking up of bacteria by substances present in certain sera; differing from proteolysis in that the bacteria may not be dead.

Chemiotaxis: the property of certain cells of being attracted towards (positive) or repelled from (negative) foreign bodies, such as bacteria, or irritant substances.

Complement: first conceived by Ehrlich, in an extension of his antitoxine theory, to account for the facts in immunity against *infection.* Exists normally in serum and is destroyed after an exposure of one-half hour to 55° C. Must act with the immune body to produce hemolysis, bacteriolysis, etc. Contains two atom-groups of affinities, one binding it to the immune body, the other exerting the active lysin action (hemolysis, bacteriolysis, etc.); this corresponds to the toxophorous atom-group in the toxines. Perhaps the same as alexines of Bordet.

Cytase: Metchnikoff's term for the substance existing in the phagocytes, which acting through, or with the immune body, destroys the bacteria. It is single of its kind; corresponds to the complement of Ehrlich, except that the latter is said to be of many kinds and to exist in the blood stream.

Enzymes: non-organized ferments, capable of causing splitting or decomposition of other substances without entering into combination with them or their products.

Fermentation: the process in which a body may originate changes in other bodies, whilst remaining unchanged itself.

Fresh animal: fresh sera, etc.; not treated in any way to produce any form of immunity.

Globulins: albuminous bodies, insoluble in water, but soluble in dilute neutral salt solutions; possibly the active principle of the antitoxines.

Go-betweens: see Zwischen-körper.

Haptines: Ehrlich's name for all the groups of receptors.

Haptophorous (binding): applied to groups of affinities in body and bacterial cells, as well as in toxines and immune bodies in general; satisfied by corresponding affinities in other bodies; receptors.

Hemolysis: breaking up of red corpuscles, see lysins. Three factors are necessary for the production of the phenomenon: 1, red-blood corpuscles to be acted upon; 2, a body occurring in the serum of immune animals and resisting heat up to 65° C., *i.e.*, the immune body; 3, a body not resisting heat above 55° C., and occurring in the blood of a fresh animal, *i.e.*, the complement.

"*Horror autotoxicus*": Ehrlich's expression for the fact that the tissues seldom, if ever, produce antibodies to receptors already existing in them.

Immune body: exists in the serum of an immunized animal; resists one-half hour at 75° C.; has two haptophorous atom-groups, one satisfied by a receptor in the cell binding them together, the other satisfied by a receptor in the complement binding these two together.

Immunity: Natural, exists in many animals to many infectious diseases.

Acquired, may be the result of an attack of a disease, or the result

of artificial procedures. If the latter, it may be

Active: the result of the use of toxines, or

Passive: the result of the use of antitoxines.

Immunity unit: see antitoxine unit.

Inactivated: used especially to denote the destruction of the complement in an immune serum by exposure to heat (55° C.), thus making the immune body inactive.

Infection: often applied to the action of the bacteria, as distinct from that of their toxines.

Intermediary body: see Immune body.

Intoxication: applied to the results of the action of bacterial poisons as distinguished from that of the bacteria themselves.

Isolysin: a substance produced artificially in serum, capable of destroying the blood corpuscles of animals of the same species.

Isopathic immunity: term suggested by Behring to indicate the immunity of the individual cell (the condition he thinks probably obtains in active immunity); immunity secured by the use of toxines.

Leucopenia: absence or disappearance from a part of all phagocytes or wandering cells; occurs temporarily in intraperitoneal injections. (Metchnikoff.)

Lysins: see Spermatolysin.

Macrocytase: cytase derived from the macrophagocytes, and active in the destruction of blood corpuscles (hemolysis). (Metchnikoff.)

Macrophagocytes: large mononuclear leucocytes of the blood and of the endothelium, and also, according to Metchnikoff, from the large cells lining the sinuses of the lymph glands and the sinuses of the spleen.

Microcytase: cytase derived from the microphagocytes, and concerned in the destruction of bacteria. (Metchnikoff.)

Microphagocytes: polymorphonuclear leucocytes.

Minimal lethal dose (*M.L.D.*): the amount of any toxine necessary to kill a two hundred and fifty gram guinea-pig in four days, after subcutaneous injection.

Passive immunity: more or less temporary immunity, the result of the use of the serum of

an animal already subjected to active immunity (antitoxine immunity).

Peritoneum: the French conception is "of a great opened-out gland (un ganglion lymphatic étalé), whose cells have proliferative and protective powers, and whose removal renders an animal more susceptible to bacterial infection than usual." (Ritchie.)

Pfeiffer's reaction (or Phenomenon): the disintegration of bacteria resulting when these, together with immune serum, are injected into the peritoneal cavity of a fresh animal. The starting point of all the recent work on bacteriolysis, hemolysis, etc.

Phagocytosis: the property possessed by certain fixed and free ameboid cells of englobing and digesting bacteria and other bodies. Supposed by Metchnikoff to be derived from the cells of the mesoderm.

Phagolysis: partial or complete destruction of phagocytes, used especially in connection with intraperitoneal injections in Pfeiffer's phenomenon. (Metchnikoff.)

Precipitines: substances found in the blood of an animal, after injection with blood of an-

other species of animal, capable of producing a precipitate in the blood of the same species of animal from which the injections were made. The precipitate found is soluble (in some cases at least) in two per cent solution of sodium chloride, and is therefore not a coagulation.

Proteolysis: the breaking up of proteid bodies as seen in ordinary digestion in connection with the intestinal glands; takes place in dead material.

Reactivated: used to denote the supplying of fresh complement to an immune serum, previously made inactive by heat (55° C.), thus making the immune body *active.* (See Inactivated.)

Receptors: chemical affinities conceived by Ehrlich as existing in the blood corpuscles, other tissue cells, etc.; satisfied by corresponding affinities in other substances (immune bodies, toxines, etc.).

Receptors of the first order: affinities in the cell concerned with fixing relatively simple ma-

terials, *i.e.*, toxines, ferments and other cellular secretions.

Receptors of the second order: affinities in the cell, fixing the molecule by one arm and breaking it up with another of a ferment-like capacity; more complicated and for absorbing molecules of large size.

Receptors of the third order: affinities fixed in the cell, and also containing two haptophorous atom-groups, one fixing the food particles, the other fixing the ferment-like body (complement), whose action is necessary for breaking up the particle fixed; the most important of the three groups.

Sensitizing substance (*substance sensibilisatrice*): see Immune body; it is the same, except that it is supposed to exist *in* the cell rather than in the blood stream.

Side-chains: see Receptors. The term "side-chains" should be used only of a molecule whose composition is known.

Spermatolysin (*and other lysins*): refer to experiments made with certain cells, after injecting which sera can be obtained contain-

ing substances capable of destroying these cells.

Stimulines: Metchnikoff's term to denote bodies existing in immune sera, which *stimulate* the phagocytes to move in a special direction, englobe, and digest invading bacteria.

Tetanus sine tetano: used by Dönitz to describe the condition of a rabbit dying after injections of tetanus toxine and antitoxine not quite balanced; death from marasmus, but no tetanus.

Tetano-lysin: a substance existing in some bouillon cultures of the tetanus bacillus, capable of destroying red-blood corpuscles of certain animals; destroyed by heat at 50° C. (Madsen.)

Tetano-spasmin: what is usually considered the tetanus toxine; with no hemolytic power; very slightly affected by twenty minutes at 50° C. (Madsen.)

Toxine immunity: active immunity.

Toxophorous (*poison carrier*): the group of chemical affinities in the toxine molecule

(and in the bacterial cell molecule in certain cases) that exerts the poisonous action on the cell, or is neutralized by the antitoxine.

Toxines: the powerful poisonous substances elaborated during the growth of many bacteria; the soluble toxines are those remaining in the filtrate of fluid cultures of some bacteria.

Toxoids: the less poisonous substances remaining in a filtered culture when this has been kept for some time, and after the soluble toxines have disappeared.

Toxones: supposed by Ehrlich to exist along with toxines and toxoids and possessing haptophorous atom-groups having a less strong affinity for similar groups in antitoxine or cell than toxine; capable, however, of combining with antitoxine, and of producing very slow poisonous results (*e.g.*, diphtheria paralyses).

Vital nucleus: Ehrlich's term for his conception of a centre of vital activity existing in the protoplasm of the cell, with which are associated the special cell capacities.

Zwischen-körper: bodies in normal sera capable of linking the hemolysin (or other lysins) to the cell; corresponding to the immune bodies in immune sera.

[NOTE. — This glossary contains terms found in general reading, as well as those in the text.]